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INCREASE IN EPITHELIAL CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE FOLLOWING VANADATE

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Vanadate increases the cyclic adenosine 3′,5′-monophosphate (cyclic AMP) content of frog skin epithelium and apparently antagonizes the stimulation by isoprenaline. The effect appears to be a direct activation of adenyl cyclase. This new effect of vanadate together with the inhibitory effects on Na-K ATPase may explain the irregular effects on sodium transport.

Introduction Following the discovery that vanadate is a potent inhibitor of membrane Na-K adenosine triphosphatase (Na-K ATPase), (Josephson & Cantley, 1977) there has been a growing interest in the actions of this anion on numerous systems. Vanadate produces a profound natriuresis in rats by a kidney action (Balfour, Grantham & Glynn, 1978) and in the skin of *Rana ridibunda* vanadate reduces sodium transport consistent with inhibition of Na-K ATPase (De Sousa & Grosso, 1979). Our results suggest that sodium transport may be affected by more than one mechanism.

Methods Sodium transport in the skin of *Rana temporaria* was measured as short circuit current (SCC) by conventional methods (Cuthbert & Fanelli, 1978). The bathing solution had the following composition (mM) NaCl 110, KCl 2.0, $CaCl_2$ 2.0, Tris buffer (pH 7.6) 5.0 and glucose 11.1.

Cyclic adenosine 3′,5′-monophosphate (cyclic AMP) in epithelium was measured by a standard protein binding radioassay (Brown, Albano, Ekins & Scherzi, 1971). Epithelia were isolated from the underlying connective tissue by collagenase and mild hydrostatic pressure (Aceves & Erlij, 1971) and discs (0.4 cm diameter) were cut out and distributed between various incubation media containing, where appropriate, vanadate or other drugs. Results are expressed as pmol cyclic AMP/cm^2 of epithelium. Vanadate (up to 1 mM) had no effect on the sensitivity of the assay.

Results The effects of vanadate (as sodium orthovanadate) added to the fluid bathing the serosal side of frog skin was investigated on 18 occasions. Provided sufficient vanadate was added the SCC, and hence sodium transport, was abolished. However a clear pattern of concentration-dependent inhibition was not found. Starting with vanadate, 1 μM, inhibition of SCC was generally observed. If the vanadate concentration was then increased in a cumulative fashion there was a surprising increase in SCC at intermediate concentrations, reversing the previous inhibition, only to be followed by further inhibition at yet higher concentrations (1 mM). We concluded that vanadate affected transport by at least two mechanisms; inhibition, probably as a result of ATPase inhibition and stimulation by an unknown mechanism. As increased cyclic AMP content is associated with hormonally stimulated transport in this tissue (Jard, 1973), we have measured cyclic AMP content in a variety of conditions with vanadate present.

In preliminary experiments we found that vanadate did indeed increase cyclic AMP content and that the effect is maximal at 30 min. Exposure of tissues to isoprenaline for 4 min was used as a hormonal stimulus, primarily to show that hormonally linked adenyl cyclase survived the collagenase treatment needed to prepare the tissue. In eight experiments, tissue cyclic AMP content increased from 0.7 ± 0.2 pmol/cm^2 to 4.6 ± 1.1 and 4.9 ± 1.5 pmol/cm^2 following 30 min exposure to 0.1 and 1.0 mM vanadate respectively. Isoprenaline (1 μM) increased cyclic AMP content to 7.3 ± 0.9 pmol/cm^2.

To investigate if the effect of vanadate was due to increased synthesis or reduced breakdown, the effects of vanadate were measured in the presence of sufficient theophylline to cause substantial inhibition of phosphodiesterase. In the presence of theophylline, 10 mM, vanadate caused a significant increase in cyclic AMP content, although significantly less than caused by isoprenaline in the same conditions (Table 1).

In further experiments the ability of vanadate to interfere with the stimulation of adenyl cyclase via the β-receptor was examined. In the presence of vanadate and isoprenaline the cyclic AMP content was significantly less than with isoprenaline alone (Table 1).

It seemed conceivable, although unlikely, that as vanadate inhibits Na-K ATPase the increase in cyclic AMP content may simply reflect the increased availability of ATP. One way to test this in intact epithelia is to incubate tissues for 1 h with ouabain 1 mM, a

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Table 1 Effects of vanadate (1 mM) on cyclic AMP content in the presence of theophylline (10 mM), isoprenaline (1 μM) and ouabain (1 mM)

Conditions	*Cyclic AMP content* (pmol/cm^2)	
Theophylline	1.27 ± 0.17	2.30 ± 0.11
Theophylline then vanadate	3.89 ± 0.33**	13.23 ± 0.84**
Theophylline then isoprenaline	8.86 ± 0.32**	18.91 ± 1.71**
Control	1.27 ± 0.09	0.53 ± 0.20
Vanadate	3.62 ± 0.82*	1.23 ± 0.07*
Isoprenaline	7.53 ± 0.41*	8.18 ± 1.72*
Vanadate then isoprenaline	5.53 ± 0.51*	1.35 ± 0.11*
Control	0.53 ± 0.02	>2.0
Ouabain	0.92 ± 0.08	2.15 ± 0.39
Vanadate	1.23 ± 0.07*	6.93 ± 0.35*
Ouabain then vanadate	1.61 ± 0.01*	4.57 ± 0.24*

Tissues were exposed to vanadate for 30 min, isoprenaline for 4 min, theophylline for 60 min and ouabain for 90 min. The incubations were carried out in the same solution used for SCC measurements and afterwards tissues were transferred to boiling Brown's buffer to stop the reaction. Means ± s.e. refer to replicate determinations (5) in single experiments. Each type of experiment was repeated twice and the results presented in separate columns. Values marked with asterisks are significantly different from controls (**$P < 0.001$; *$P < 0.025$). Bracketed values are significantly different at $P < 0.02$.

procedure known to inhibit transport completely and hence increase the ATP supply. Vanadate was able to increase cyclic AMP content significantly in tissues pretreated with ouabain in this way (Table 1).

Discussion At present the most consistent interpretation of the data presented is that vanadate stimulates adenyl cyclase by a direct action. It may well be, but we have not proved, that the stimulation of SCC caused by vanadate is by an effect on adenyl cyclase and that the irregular pattern of response in intact epithelia is due to a combination of ATPase inhibition and adenyl cyclase activation. The antagonism by vanadate of isoprenaline effects on transport parameters reported by De Sousa & Grosso (1979) is, from our results, a likely consequence of vanadate affecting a receptor-adenyl cyclase interaction. It is possible that vanadate reduced the effectiveness of isoprenaline by promoting its oxidation but from the data of Krawietz, Werdman & Erdmann (1979) this seems unlikely.

While this study was in progress two publications appeared showing that vanadate can stimulate adenyl cyclase in non-epithelial tissues, fat cells and cardiac muscle (Schwabe, Puchstein, Hannemann & Sochtig, 1979; Krawietz *et al.*, 1979). In both instances the effective concentration was similar to that reported here and higher than that required for ATPase inhibition. In cardiac muscle it is suggested that the effect on cyclic AMP content may be related to the inotropic response to vanadate. In fat cells the effects of noradrenaline and vanadate are not additive as was found for the vanadate isoprenaline interaction in this study, while in cardiac muscle isoprenaline and vanadate appear to stimulate the enzyme independently.

It would appear, therefore, that the stimulation of adenyl cyclase by vanadate may be a rather general phenomenon, and a very recent report indicates that this may extend to the molybdate ion too (Richards & Swislocki, 1979).

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